AWARD NUMBER: W81XWH-13-1-0253

TITLE: Brain and Plasma Molecular Characterization of the Pathogenic TBI-AD Interrelationship in Mouse Models

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REPORT DATE: October 2015

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
October 2015	Annual	16Sep2014 - 15Sep2015

4. TITLE AND SUBTITLE

Brain and Plasma Molecular Characterization of the Pathogenic TBI-AD Interrelationship in Mouse Models

5a. CONTRACT NUMBER
W81XWH-13-1-0253

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Roskamp Institute
Sarasota, FL 34243

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT **Traumatic Brain Injury (TBI), in particular mild TBI (mTBI) is a major cause of disability in military and in civilian populations, and for many years has been known to be an epigenetic risk factor for Alzheimer's Disease (AD) and other neurodegenerative conditions such as Parkinson's disease, Amyotrophic Lateral Sclerosis or Chronic Traumatic Encephalopathy (CTE) (Randolph, 2001; Gaetz and Weinberg, 2000; Gaetz et al., 2000; Guskiewicz et al., 2003; Gavett et al., 2011; Van Den Heuvel et al., 2007a; Gavett et al., 2010; Förstl et al., 2010; McKee et al., 2009; Costanza et al., 2011; Stern et al., 2011). The existence of a TBI-AD relationship is well recognized (Guo et al., 2000; Jellinger et al., 2001; Mauri et al., 2006; Mayeux et al., 1993; Mayeux et al., 1995; Schofield et al., 1997; Tang et al., 1996; Van Den Heuvel et al., 2007b; Blyth and Bazarian, 2010; Członkowska and Kurkowska-Jastrzębska, 2011; Hinkebein et al., 2003; Sivanandam and Thakur, 2012; Verghese et al., 2011), and the overlaps and distinctions between pathological features of AD and TBI, including (more recently) CTE, have long been the subject of reporting and discussion (Tokuda et al., 1991; Graham et al., 1995; Irving et al., 1996; Geddes et al., 1996; Geddes et al., 1999; Jellinger et al., 2001; Schmidt et al., 2001; Smith et al., 2003; Uryu et al., 2007; Dekosky et al., 2007; McKee et al., 2009; Magnoni and Brody, 2010; Johnson et al., 2011; Magnoni et al., 2011; Shively et al., 2012; Johnson et al., 2013; McKee et al., 2013; Omalu et al., 2011). However, the precise nature of how TBI leads to or precipitates AD pathogenesis is not understood. The goal of this proposal is to use molecular level approaches in relevant animal models to identify overlapping profiles between TBI and AD and determine early critical cellular responses to TBI that can be targeted for therapeutic intervention. By identifying the molecules and pathways that are common to both AD and TBI mouse models we will highlight cellular mechanisms that represent critical steps relating TBI to AD.**

15. SUBJECT TERMS

Nothing listed

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
Unclassified	Unclassified	Unclassified	U	UU	19b. TELEPHONE NUMBER *(include area code)*

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1 Introduction

Traumatic Brain Injury (TBI), in particular mild TBI (mTBI) is a major cause of disability in military and in civilian populations, and for many years has been known to be an epigenetic risk factor for Alzheimer's Disease (AD) and other neurodegenerative conditions such as Parkinson's disease, Amyotrophic Lateral Sclerosis or Chronic Traumatic Encephalopathy (CTE) (Randolph, 2001; Gaetz and Weinberg, 2000; Gaetz et al., 2000; Guskiewicz et al., 2003; Gavett et al., 2011; Van Den Heuvel et al., 2007a; Gavett et al., 2010; Förstl et al., 2010; McKee et al., 2009; Costanza et al., 2011; Stern et al., 2011). The existence of a TBI-AD relationship is well recognized (Guo et al., 2000; Jellinger et al., 2001; Mauri et al., 2006; Mayeux et al., 1993; Mayeux et al., 1995; Schofield et al., 1997; Tang et al., 1996; Van Den Heuvel et al., 2007b; Blyth and Bazarian, 2010; Członkowska and Kurkowska-Jastrzębska, 2011; Hinkebein et al., 2003; Sivanandam and Thakur, 2012; Verghese et al., 2011), and the overlaps and distinctions between pathological features of AD and TBI, including (more recently) CTE, have long been the subject of reporting and discussion (Tokuda et al., 1991; Graham et al., 1995; Irving et al., 1996; Geddes et al., 1996; Geddes et al., 1999; Jellinger et al., 2001; Schmidt et al., 2001; Smith et al., 2003; Uryu et al., 2007; Dekosky et al., 2007; McKee et al., 2009; Magnoni and Brody, 2010; Johnson et al., 2011; Magnoni et al., 2011; Shively et al., 2012; Johnson et al., 2013; McKee et al., 2013; Omalu et al., 2011). However, the precise nature of how TBI leads to or precipitates AD pathogenesis is not understood. The goal of this proposal is to use molecular level approaches in relevant animal models to identify overlapping profiles between TBI and AD and determine early critical cellular responses to TBI that can be targeted for therapeutic intervention. By identifying the molecules and pathways that are common to both AD and TBI mouse models we will highlight cellular mechanisms that represent critical steps relating TBI to AD.

2 Keywords

Proteomic; Lipidomic; Alzheimer's Disease (AD); mild Traumatic Brain Injury (mTBI); mouse models; pathogenesis; molecular profiles.

3 Overall Project Summary

Our team has a long and productive history of research into Alzheimer's Disease, including identification of the first human AD-causing mutations (which enabled the creation of animal models of the disease) (Goate et al., 1991; Chartier-Harlin et al., 1991; Mullan et al., 1992) and identification of a novel therapeutic target for AD which has resulted in human clinical trials for a novel treatment (Paris et al., 2014; Kennelly et al., 2011a; Kennelly et al., 2011b) and the European Phase III NILVAD trial (Lawlor et al., 2014). We have also developed a novel mouse model of mild TBI (mTBI)/concussion in which we have demonstrated cognitive dysfunction at 6, 12, 18 and 24 months after a repetitive TBI and neuropathological changes consistent with those observed in human TBI patients (Mouzon et al., 2012; Mouzon et al., 2013; Ojo et al., 2013; Tzekov et al., 2014). We have established a proteomic and lipidomic platform with which we have characterized brain and plasma responses in mouse models of TBI, AD and other neurodegenerative conditions (Abdullah et al., 2014; Abdullah et al., 2013; Crawford et al., 2012; Abdullah et al., 2012; Abdullah et al., 2011).

The goal of this project is to generate a detailed timecourse of the molecular changes in laboratory models of AD and mTBI in order to determine the critical sequelae of mTBI that lead to, and overlap with, AD pathogenesis. For our Alzheimer's mouse models we are using the PSAPP mouse model of AD, expressing the PS1(M146L) and APP(K670N,M671L) mutations (Schuessel et al. 2006; Trinchese et al. 2004; Sadowski et al. 2004; Wengenack et al. 2000; Holcomb et al. 1998), and the hTau mouse model which expresses all six isoforms of human tau on a null murine tau background (Andorfer et al. 2003, 2005; Polydoro et al. 2009) and relevant strain controls (B6SJL and C57BL/6). For our TBI work we are also using the hTau mice, in order to explore tau specific changes, and strain controls (C57BL/6).

Tasks

Task 1: To identify age/time-dependent expression of brain proteins and lipids in mouse models of AD (PSAPP and hTau) and of mTBI (single and repetitive mTBI in hTau and wild type) compared to control mice.
SubTask 1a: Proteomic and lipidomic characterization of hippocampi and cortices from AD mice and strain controls at 3, 9 and 15 months of age (representing timepoints of pre-, developing and established pathology). 96 mice will be required for this subtask.
SubTask 1b: Proteomic and lipidomic characterization of hippocampi and cortices from mTBI mice and anesthesia controls at 24hrs, 3, 6, 9 and 12 months after single or repetitive mTBI or anesthesia. 320 mice will be required for this subtask.
Deliverable 1: Molecular profiles of response to AD pathogenesis and mTBI in mouse models.

Task 2: To identify areas of molecular overlap in the brains' response to mild TBI (mTBI) and the onset of Alzheimer's-like pathogenesis in mouse models.
SubTask 2a: Neuropathological analysis of the brains of mice from all study groups in Task 1.
SubTask 2b: Expert evaluation of the molecular profiles generated in Task 1, and the cellular pathways shown to be modulated, together with neuropathological correlates.
Deliverable 2: Critical molecular profiles and pathways in the pathogenic TBI/AD interrelationship.

Task 3: To identify blood biomarkers of the TBI/AD interrelationship.
SubTask 3a: Proteomic and lipidomic plasma analyses from TBI and AD mice (from Task 1 above)
SubTask 3b: Identification of plasma biomarker profiles that overlap between TBI and AD models and that correlate with convergence of brain TBI and AD molecular profiles.
Deliverable 3: Putative blood biomarkers of TBI/AD for further exploration and validation in human studies.

Current status

Despite initial delays in establishing the many different cohorts for these studies, and the tight timeline necessitated by the plan to investigate aged AD mice and TBI mice at extended time points post-injury, we successfully populated all groups and completed all euthanasias and tissue collection within the two year timeframe for the contract.

AD models	3 months old	9 months old	15 months old
PSAPPsw	X	X	X
hTau	X	X	X
C57BL6 (control for both models)	X	X	X

TBI models	Acute post-TBI	3 mo. post TBI	6 mo. post TBI	9 mo. post TBI	12 mo.post TBI
C57BL6 s-sham	X	X	X	X	X
C57BL6 s-mTBI	X	X	X	X	X
C57BL6 r-sham	X	X	X	X	X
C57BL6 r-mTBI	X	X	X	X	X
hTau s-sham	X	X	X	X	X
hTau s-mTBI	X	X	X	X	X
hTau r-sham	X	X	X	X	X
hTau r-mTBI	X	X	X	X	X

The first year was largely focussed on generation of the required animal colonies and development and honing of the methods and analytical tools for omic data analysis, but lipidomic data generation did begin as discussed below.
Owing to the planned plex-assignment for samples for proteomic analyses, enabling direct comparison of relevant sample groups, proteomic data analyses had to wait for completion of all groups and is currently underway during our no-cost extension.

Proteomics Experimental Design and Analysis Plan (Ongoing)

In the mTBI study wildtype and hTau mice receive injury (single sham, repetitive sham, single mTBI and repetitive mTBI) at 10-12 weeks of age, and are euthanized for sample collection at 5 different timepoints after the last injury (24hrs, 3, 6, 9 and 12 months). In the AD study samples from PSAPP mice, hTau mice and mice from their background strains will be collected at three different ages (3, 9 and 15 months). Since the total number of animals and of samples is high, the most efficient way of surveying the proteome is a multiplexed approach such as Tandem Mass Tag™ (TMT). However TMT, and other proteome surveying approaches, are relative quantification techniques (unlike the lipidomic approach we are

using), therefore the samples that are on the same plex can be compared to each other but across plex comparison is only possible through ratios that are formed with the samples within the same plex. Thus planning the appropriate sample grouping on the plexus is an important consideration.

In the mTBI cohorts the main goal is to capture response to injury in wild type and hTau mice over five different timepoints. Therefore all timepoints for sham and mTBI injured animals for a given genotype should be on the same plex, requiring use of the TMT 10-plex. Each plex will contain one sample from each of:

i C57BL6 mice receiving s-sham or s-mTBI and euthanized at 24hr, 3, 6, 9 or 12 months post injury.

ii hTau mice receiving s-sham or s-mTBI and euthanized at 24hr, 3, 6, 9 or 12 months post injury.

iii C57BL6 mice receiving r-sham or r-mTBI and euthanized at 24hr, 3, 6, 9 or 12 months post injury.

iv hTau mice receiving r-sham or r-mTBI and euthanized at 24hr, 3, 6, 9 or 12 months post injury.

As there are 4 mice per group this will require 16 10-plexes for each tissue type under investigation (plasma, hippocampi, cortices) to complete the mTBI analyses..

The ratio of mTBI/Sham will be determined at each timepoint for a given genotype and injury type (single or repetitive). Identification of time-dependent changes to all the quantified proteins will be facilitated by having 5 timepoints from a given genotype and injury type on the same 10-plex see **Figure 1**.

mTBI cohorts

hTau 10-plex	hTau 24 hrs s-sham	hTau 3 M s-sham	hTau 6 M s-sham	hTau 9 M s-sham	hTau 12 M s-sham	hTau 24hrs s-mTBI	hTau 3M s-mTBI	hTau 6M s-mTBI	hTau 9 M s-mTBI	hTau 12 M s-mTBI
C57BL6 10-plex	C57BL6 24 hrs s-sham	C57BL6 3 M s-sham	C57BL6 6 M s-sham	C57BL6 9 M s-sham	C57BL6 12 M s-sham	C57BL6 24 hrs s-mTBI	C57BL6 3 M s-mTBI	C57BL6 6 M s-mTBI	C57BL6 9 M s-mTBI	C57BL6 12 M s-mTBI
hTau 10-plex	hTau 24 hrs r-sham	hTau 3 M r-sham	hTau 6 M r-sham	hTau 9 M r-sham	hTau 12 M r-sham	hTau 24hrs r-mTBI	hTau 3M r-mTBI	hTau 6M r-mTBI	hTau 9 M r-mTBI	hTau 12 M r-mTBI
C57BL6 10-plex	C57BL6 24 hrs r-sham	C57BL6 3 M r-sham	C57BL6 6 M r-sham	C57BL6 9 M r-sham	C57BL6 12 M r-sham	C57BL6 24 hrs r-mTBI	C57BL6 3 M r-mTBI	C57BL6 6 M r-mTBI	C57BL6 9 M r-mTBI	C57BL6 12 M r-mTBI

Figure 1. In order to capture response to injury (s-mTBI/s-sham or r-mTBI/r-sham) over time in the hTau transgenic mouse line and its own background strain TMT 10-plexes will be used as shown.

In the AD cohort the main goal is to measure age-dependent changes in the proteome of "diseased" versus control mice, therefore it is only meaningful to compare PSAPP to its background strain (B6SJL) and hTau to its background strain C57BL6 at the three time points. As the proteomic response to progression of disease is also critical for us to capture, in order to map TBI-dependent changes against AD progression, all the timepoints for given transgenic animal strain and its background strain should be on the same multi-plex. Thus we propose the use of TMT 6-plex for the AD proteomic analyses – each plex containing one sample from each of:

i PSAPP and B6SJL aged 3 months; PSAPP and B6SJL aged 9 months; and PSAPP and B6SJL aged 15 months or

ii hTau and C57BL6 aged 3 months; hTau and C57BL6 aged 9 months; and hTau and C57BL6 aged 15 months

The ratios of protein expression in AD model versus strain control will be used to capture PSAPP or hTau dependent changes to the proteome over time. Having all time points on the same multiplex will also enables observation of a single protein over time. With 4 mice per group this will require 8 6-plexes for each tissue type under investigation (plasma, hippocampi, cortices) to complete the AD analyses (**Figure 2**).

AD cohorts

PSAPP 6-plex	PSAPP 3 Month	PSAPP 9 Month	PSAPP 15 Month	B6SJL 3 Month	B6SJL 9 Month	B6SJL 15 Month
hTau 6-plex	hTau 3 Month	hTau 9 Month	hTau 15 Month	C57BL6 3 Month	C57BL6 9 Month	C57BL6 15Month

Figure 2. In order to capture disease progression in PSAPP and hTau transgenic mouse lines over time, for each model (PSAPP or hTau) we will analyze all three ages in the transgenic and the strain control together, using TMT 6-plex.

4 Key Research Accomplishments

During this fourth quarter, and throughout this year, we have populated cohorts of animals, administered injuries/sham injuries and aged mice to the relevant time points such that at the conclusion of this second year all cohorts have been populated and have reached their time points for euthanasia and have been euthanized with relevant tissue collection.

Lipidomic Data generated in Y2

Lipidomic data generation has been ongoing throughout this year as each cohort relevant for direct comparison was completed, since lipid data are absolute measurements against internal standards as compared to the proteomic analyses which are relative values.
Our **Y2Q1** report comprised our presentation at the January PLR which reported plasma lipidomic analyses from the wild type s-sham, s-mTBI, r-sham, r-mTBI model at 24hrs, 3 months and 6 months post injury, and also from the AD hTau model and strain control at 3 and 9 months of age. We showed that for plasma PC and LPC there were increases in particular lipid species in response to TBI. For the hTau mice compared to controls we observed age dependent differences in LPC species. Preliminary analyses of cortical and hippocampal tissue from the r-mTBI and r-sham WT mice showed significant decreases in hippocampal lipid PC species and trends for decreases in the cortices.
By **Y2Q2** we extended our plasma analyses to include later time points to 24 months post injury in the wild type mice. Discrepancies with our plasma lipid data prompted reevaluation of our approach which was addressed in **Y2Q3** wherein we described the change from in-source collision induced dissociation (SCID) based liquid chromatography/mass spectrometry (LC/MS) method to hydrophilic Interaction liquid chromatography (HILIC), using hydrophilic stationary phases with reverse phase type solvents allowing for the elution of phospholipids in the order of increasing polarity of the head-group. This method allows us to detect and quantify highly polar quaternary-amine containing PLs in the negative ion scanning mode, which is how the remainder of PL classes in this assay are also detected and quantified. In addition, a major advantage of this HILIC method is that PC, SM, and LPC quantification are not interfered with by sodium and potassium adducts. An additional quality-control measure recently introduced during data analysis is implementing background subtraction when processing the raw data. Hence, with implementation of new LC/MS parameters and methods as well as additional quantify control measures during processing of raw data, we are able to keep the intra-assay variability as low as 10-15% of coefficient of variance. Lipidomics platforms are still a “work in progress” in the research arena, both in terms of technical approaches and data interpretation. All of our lipidomic data are now being/have been reanalyzed using the HILIC approach. Under this methodology our analysis of brain tissue responses reported in **Y2Q3** showed increases in particular lipid species in the cortex of injured versus sham mice, and decreases in the hippocampi. However, we also found significant decreases in some lipid species in the cortex. In hTau versus wild type mice we observed some significant increases in cortical lipids versus sham at 3, 9 and 15 months of age.

Lipidomic Data generated in Y2Q4

In addition to absolute changes in lipid levels we are interested in the arachidonic acid containing lipids and the docosahexaenoic acid containing lipids and their ratio, as there is now substantial evidence that an imbalance in AA and DHA may contribute to the neuroinflammatory processes within the brain. For instance, AA is predominantly converted to eicosanoids by cyclooxygenase and lipoxygenase, which mediate a host of pro-inflammatory processes. However, processing of DHA by these lipoxygenases yields anti-inflammatory bioactive lipid metabolites, such as protectin, that can resolve inflammation even at low nanomolar concentrations (Serhan and Chiang, 2008). We therefore examined the ratios of AA and DHA containing PE and PC species as they represent a rich source of these essential polyunsaturated fatty acids (PUFA). We observed that the ratios of AA- to DHA-containing PE species from the cortices of wild-type mice *decrease* with age. However, when comparing wild-type injured mice to wild-type sham mice the ratios of AA to DHA containing species were increased at 3- and 6-months post-injury. PSAPP mice showed similar decreases with age in the ratios of AA and DHA-containing PE species as observed in their wild-type littermates. However, in hTau mice, there was a significant increase in the ratio of AA to DHA containing PE species at 6-months of age, a timepoint which corresponds with the data from 3-months post-injury in wild-type mTBI mice. We next examined the ratios of AA- to DHA-containing PC species. As with PE, wild-type mice had significant decreases in AA to DHA ratios within the PC class with age but the levels were elevated in injured relative to sham mice at 3- and 6-months post-injury. For PSAPP mice, there were significant *increases* in AA to DHA ratios for PC when comparing 3-month old PSAPP mice to 9 and 15-month old wild-type mice. An elevated AA to DHA ratio was also noted when comparing 15-month old PSAPPswe to 15-month old wild-type mice. <u>These findings suggest that an increase in ratios of AA to DHA within PL species might be a common pathogenic feature between mTBI and AD</u>, and these may implicate an increase in the production of inflammatory eicosanoids such as leukotrienes.

Figures 3 & 4: **A ratio of AA to DHA containing PLs is elevated in TBI and also in AD mouse models**. Mean ± SE (n = 4 per group). Following LC/MS analysis, identifications of AA and/or DHA containing PE (**Fig 3**) and PC (**Fig 4**) species were confirmed using LC/MS/MS (or using Lipid Maps databases where applicable). (3a) AA and DHA species decrease with age in wild-type mice. (3b) In wild-type mice following mTBI, ratios of AA to DHA were higher at 3- and 6-months post-injury. (3c) In PSAPP mice, ratios of AA to DHA containing PE decreased with age but these differences were not statistically significant. (3d) In hTau mice, the ratios of AA to DHA within PE were elevated at 6-months of age, corresponding with 3-months post-injury. (4a) As with PE, ratios of AA to DHA in PC class were lower with age. (4b) In wild-type mice following injury, AA to DHA ratios in PC were elevated at 3- and 6-months post-injury. (4c) 3-month old PSAPP mice also had higher AA to DHA ratios in PC compared to 6- and 9-months old wild-type mice. At 15-months of age PSPAPP mice had higher AA to DHA ratios within PC compared to 15-months old wild-type mice (4d) There were no significant differences in the AA to DHA ratio in hTau mice between 3 to 15 months of age. $^*p < 0.05$.

Figure 3a-d AA:DHA Phosphatidylethanolamine (PE)

Fig 3a

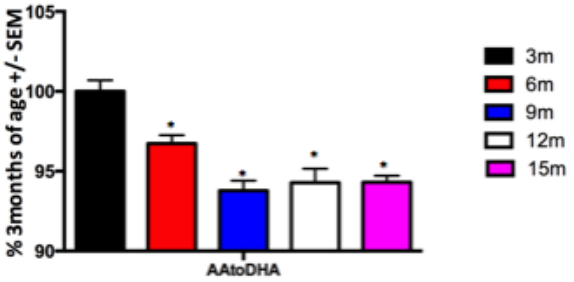


Fig 3b

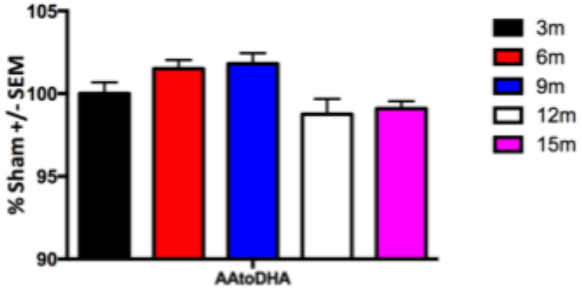


Fig 3c

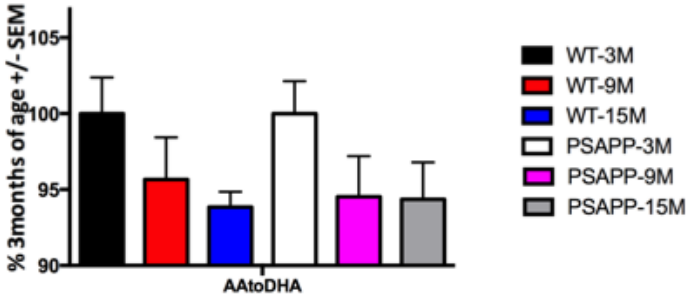


Fig 3d

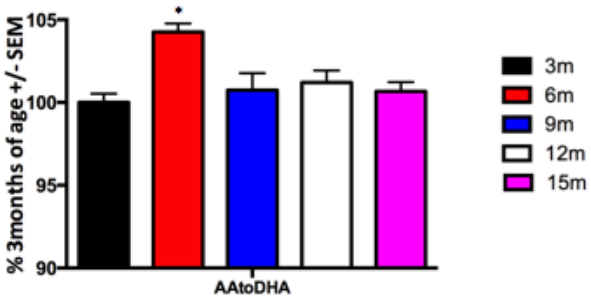


Figure 4a-d AA:DHA Phosphatidylcholine (PC)

Fig 4a

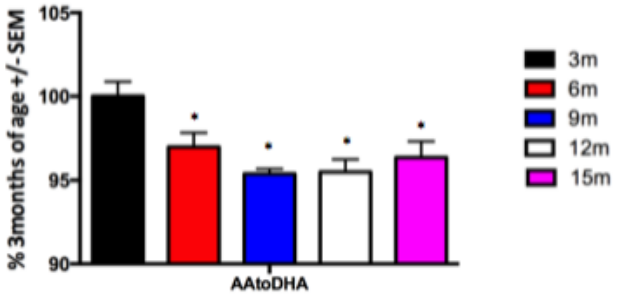


Fig 4b

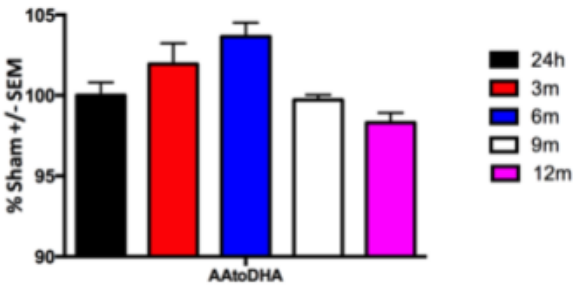


Fig 4c

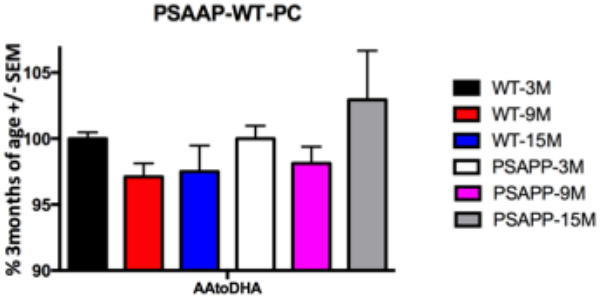
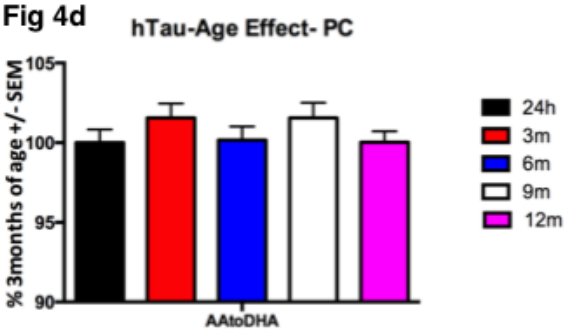


Fig 4d



Proteomic Data generated in Y2Q4

In the final quarter of Year 2 we were able to begin proteomic analyses of the r-sham and r-mTBI wild type mice (all five timepoints have been completed for this genotype) and of the hTau mouse model of AD (all three ages having been completed for both hTau and strain controls). **Table 1** shows the significantly regulated proteins in the mTBI model (mTBI vs Sham for each timepoint) and **Table 2** the significantly regulated proteins in the hTau model of AD (hTau vs WT at each age).

Table 1 - significantly regulated proteins over 12 months post-injury in wild type mice

Master Pro	Source	Description	Mean(24H	Mean(3M	Mean(6M	Mean(9M	Mean(12M
P68510	TIMEPOINT POST INJURY	14-3-3 protein eta OS=Mus musculus GN=Ywhah PE=1 SV=2	-0.05	0.19	0.87	-0.30	-0.25
P63101	TIMEPOINT POST INJURY	14-3-3 protein zeta/delta OS=Mus musculus GN=Ywhaz PE=1 SV=1	-0.03	0.26	0.50	-0.01	0.16
P16330	TIMEPOINT POST INJURY	2',3'-cyclic-nucleotide 3'-phosphodiesterase OS=Mus musculus GN=Cnp PE=1 SV=3	0.11	-0.12	-0.13	0.23	-0.19
P60710	TIMEPOINT POST INJURY	Actin, cytoplasmic 1 OS=Mus musculus GN=Actb PE=1 SV=1	0.08	0.07	0.26	0.02	-0.08
Q91XV3	TIMEPOINT POST INJURY	Brain acid soluble protein 1 OS=Mus musculus GN=Basp1 PE=1 SV=3	0.25	0.39	0.15	0.18	0.59
P40240	TIMEPOINT POST INJURY	CD9 antigen OS=Mus musculus GN=Cd9 PE=1 SV=2	-0.14	-0.06	-0.07	0.22	0.02
P16858	TIMEPOINT POST INJURY	Glyceraldehyde-3-phosphate dehydrogenase OS=Mus musculus GN=Gapdh PE=1 SV=2	0.01	0.08	0.47	0.27	0.28
P62806	TIMEPOINT POST INJURY	Histone H4 OS=Mus musculus GN=Hist1h4a PE=1 SV=2	-0.21	0.31	0.19	-0.58	-0.07
P04370	TIMEPOINT POST INJURY	Myelin basic protein OS=Mus musculus GN=Mbp PE=1 SV=2	0.09	0.14	-0.16	0.31	-0.24
P60202	TIMEPOINT POST INJURY	Myelin proteolipid protein OS=Mus musculus GN=Plp1 PE=1 SV=2	0.09	-0.28	-0.15	0.29	-0.44
O09111	TIMEPOINT POST INJURY	NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 11, mitochondrial OS=Mus musculus GN=Ndufb11 PE=1 SV=2	0.01	-0.11	-0.38	0.07	0.06
P17742	TIMEPOINT POST INJURY	Peptidyl-prolyl cis-trans isomerase A OS=Mus musculus GN=Ppia PE=1 SV=2	-0.06	-0.10	0.60	-0.11	0.28
P52480	TIMEPOINT POST INJURY	Pyruvate kinase PKM OS=Mus musculus GN=Pkm PE=1 SV=4	0.02	-0.12	0.54	0.11	0.06
P50396	TIMEPOINT POST INJURY	Rab GDP dissociation inhibitor alpha OS=Mus musculus GN=Gdi1 PE=1 SV=3	-0.03	-0.09	0.52	-0.06	0.05
P14094	TIMEPOINT POST INJURY	Sodium/potassium-transporting ATPase subunit beta-1 OS=Mus musculus GN=Atp1b1 PE=1 SV=1	0.05	0.07	-0.17	0.07	0.06

Table 2 - significantly regulated proteins up to 15 months of age in the hTau mouse model

Master Pro	Source	Description	Mean(3M	Mean(9M	Mean(15M
O09111	AGE MONTHS	NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 11, mitochondrial OS=Mus musculus GN=Ndufb11 PE=1 SV=2	-0.15	-0.33	0.05
P10636	GENOTYPE	Microtubule-associated protein tau OS=Homo sapiens GN=MAPT PE=1 SV=5	4.28	3.13	4.01
P16330	AGE MONTHS	2',3'-cyclic-nucleotide 3'-phosphodiesterase OS=Mus musculus GN=Cnp PE=1 SV=3	0.03	-0.02	0.04
P56383	AGE MONTHS	ATP synthase F(0) complex subunit C2, mitochondrial OS=Mus musculus GN=Atp5g2 PE=2 SV=2	0.04	0.15	0.18
P56391	AGE MONTHS	Cytochrome c oxidase subunit 6B1 OS=Mus musculus GN=Cox6b1 PE=1 SV=2	-0.33	-0.13	0.09
P60202	AGE MONTHS	Myelin proteolipid protein OS=Mus musculus GN=Plp1 PE=1 SV=2	0.48	-0.16	0.28
Q03265	AGE MONTHS	ATP synthase subunit alpha, mitochondrial OS=Mus musculus GN=Atp5a1 PE=1 SV=1	-0.02	-0.03	0.02
Q60692	AGE MONTHS	Proteasome subunit beta type-6 OS=Mus musculus GN=Psmb6 PE=1 SV=3	0.44	0.40	0.07
Q60932	AGE MONTHS	Voltage-dependent anion-selective channel protein 1 OS=Mus musculus GN=Vdac1 PE=1 SV=3	-0.05	-0.18	0.08
Q61885	AGE MONTHS	Myelin-oligodendrocyte glycoprotein OS=Mus musculus GN=Mog PE=1 SV=1	0.24	0.05	-0.05
Q8CAQ8	AGE MONTHS	MICOS complex subunit Mic60 OS=Mus musculus GN=Immt PE=1 SV=1	0.15	0.30	0.07
Q9CQ69	AGE MONTHS	Cytochrome b-c1 complex subunit 8 OS=Mus musculus GN=Uqcrq PE=1 SV=3	-0.48	-0.17	-0.09
Q9CQQ7	AGE MONTHS	ATP synthase F(0) complex subunit B1, mitochondrial OS=Mus musculus GN=Atp5f1 PE=1 SV=1	0.13	-0.12	-0.02
Q9DBG3	AGE MONTHS	AP-2 complex subunit beta OS=Mus musculus GN=Ap2b1 PE=1 SV=1	-0.23	-0.23	-0.09

The number of proteins detected in this experiment were lower than what is typically observed in similar samples. Upon investigation, it was concluded that the samples were diluted and this event lead to identification of only highly abundant proteins. Although the TMT labeling and MS experiments were performed well according to our quality control criteria, there was not enough sample to detect the low abundance proteins that are usually biologically very interesting. We believe that the statistically significant changes in the short list of proteins that were identified from the first run are valid, since htau protein was identified as the only protein with levels changing significantly across genotypes. Also several other proteins that were changing in both cohorts due to aging were related with functions that were relevant to aging. However, in order to get the full picture, the samples need to be re-run so that low abundance proteins can be identified and quantified in both cohorts.

5 Conclusions

Our data continue to support lipid dysregulation after TBI and in response to AD, but a complete analyses of all cohorts will be necessary in order to interpret how these changes relate to one another and to pathogenicity.

6 Publications, Abstracts and Presentations

Exploring the interrelationships between Alzheimer's disease and traumatic brain injury using Omic technologies. Ojo JO, Abdullah L, Emmerich T, Reed J, Evans J, Crynen G, Mouzon B, Mullan M, Crawford F. The Annual Society of Neuroscience meeting in Washington D.C. on November 17, 2014.

Exploring the molecular overlap in the brain and plasma of repetitive TBI and AD mouse models using proteomic technology. Ojo JO, Reed J, Evans J, Crynen G, Mouzon B, Mullan M, Crawford F. The Annual Society of Neuroscience meeting in Chicago. on October 19, 2015.

Exploring The Molecular Overlap In The Brain Of Repetitive MTBI and PSAPP Mouse Models Using Proteomics Technology. Ojo JO, Reed J, Crynen G, Ajoy R, Algamal M, Vallabhaneni P, Leary P, Mullan M, Crawford F. *Upcoming* International Neurotrauma Society meeting in Capetown on February 3, 2016

7 Inventions, Patents and Licenses

Nothing to report

8 Reportable Outcomes

a All cohorts (TBI and AD) fully populated and complete (euthanized)
b Lipidomic data generated for all samples; data analysis ongoing
c Proteomic data generation initiated

9 Other Achievements

Nothing to report

10 References

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